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(54) Monoclonal antibodies to human gastrointestinal cancer.

(57) Diagnostic panels for human gastrointestinal abnormalities such as cancer using mouse monoclonal antibodies are disclosed. These panels can be used in diagnosis and in therapeutic applications such as colon cancer.

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1 This invention was partially made with United States
government support under CA 08748 awarded by the National
Cancer Institute. The government has certain rights in this
invention.

Background

10 This invention concerns monoclonal antibodies
recognizing human gastrointestinal (GI) cells. The
monoclonal antibodies recognize antigenic markers on normal
as well as cancerous GI cells. Capable of distinguishing
among normal GI cells as well as colon carcinomas, these
mAbs are useful in diagnosis and prognosis of colon and
15 gastrointestinal cancer. Examination of human GI specimens:
tissues wastes, exudates, and fluids with these mAbs is a
diagnostic procedure to probe for cancer of the
gastrointestinal tract and especially colon cancer. These
mAbs are of special importance because of the widespread
20 occurrence of colon and stomach cancer.

In 1975 Köhler and Millstein introduced a
procedure for the production of monoclonal antibodies (mAbs)
using hybrid cells (hybridomas) which allows the production
25 of almost unlimited quantities of antibodies of precise and
reproducible specificity. Conventional antisera, produced

1 by immunizing animals with tumor cells or other antigens,
contain a myriad of different antibodies differing in their
specificity and properties, whereas hybridomas produce a
single antibody with uniform characteristics. The
5 Kohler-Millstein procedure entails the fusion of spleen
cells from an immunized animal with an immortal myeloma cell
line. From the fused cells (hybridomas), clones are
selected that produce antibody of the desired specificity.
Each clone continues to produce only that one antibody. As
10 hybridoma cells can be cultured indefinitely (or stored
frozen in liquid nitrogen), a constant supply of antibody is
assured.

15 Antibodies are proteins that have the ability to
combine with and recognize other molecules, known as
antigens. Monoclonal antibodies are no different from other
antibodies except that they are very uniform in their
properties and recognize only one antigen or a portion of an
antigen known as a determinant.
20

In the case of cells, the determinant recognized is an
antigen on or in the cell which reacts with the antibody.
It is through these cell antigens that a particular antibody
recognizes, i.e. reacts with, a particular kind of cell.
25 Thus the cell antigens are markers by which the cell is
identified.

These antigenic markers may be used to observe the normal process of cell differentiation and to locate abnormalities within a given cell system. The process of differentiation is accompanied by changes in the cell surface antigenic phenotype, and antigens that distinguish cells belonging to distinct differentiation lineages or distinguish cells at different phases in the same differentiation lineage may be observed if the correct antibody is available. Initial recognition of differentiation antigens came about through analysis of surface antigens of T-cell leukemias of the mouse and the description of the TL, Thy-1, and Lyt series of antigens. (Old, Lloyd J., Cancer Research, 41, 361-375, February 1981) The analysis of these T-cell differentiation antigens was greatly simplified by the availability of normal T cells and B cells of mouse and man and is relatively advanced. (See Patents #4,361,549-550; #4,364,932-37 and #4,363,799 concerning mAb to Human T-cell antigens). There is further experimentation to be done concerning differentiation antigens displayed on normal and neoplastic cells belonging to other lineages.

The preparation of hybrid cell lines can be successful or not depending on such experimental factors as nature of the inoculant, cell growth conditions, hybridization

4
1 conditions etc. Thus it is not always possible to predict
successful hybridoma preparation with one cell line although
success may have been achieved with another cell line.

5 Progress in defining surface antigens on melanocytes
was made possible by the recently discovered technique of
culturing melanocytes from normal skin (Eisinger, et al.,
Proc. Nat'l. Acad. Sci. USA, 79 2018 (March 1982). This
method provides a renewable source of proliferating cells
10 for the analysis of melanocyte differentiation antigens.
Likewise, a large number of cell lines derived from
melanomas have now been established and these have
facilitated the analysis of melanoma surface antigens. The
advent of mAbs has greatly accelerated knowledge about the
15 surface antigens of malignant melanoma. Cell markers on
both melanomas and melanocytes have been identified. A
panel of typing monoclonal antibodies has been selected
which recognizes differentiation antigen characteristics at
each stage of development in both melanocytes and melanomas.
20 These differentiation antigens may be used to classify
melanocytes and melanomas and to group them into
characteristic sub-sets. Dippold et al. Proc. Nat'l. Acad.
Sci. U.S.A. 77, 6114 (1980) and Houghton, et al. J. Exp.
Med. 156, 1755 (1982). Immunoassay of melanocytes and
25 melanoma cells within sub-sets is thus made possible.

Summary

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Cancers of the gastrointestinal tract are especially widespread; stomach cancer in Japan, colon cancer in the west and U.S.A. Early diagnosis would be desirable to prevent loss of life and prescribe alternatives to drastic surgery. Positive diagnosis can help to support the surgical decision. Cytohistological methods to date are not always successful. A panel group of mAbs of the present invention recognizing cancerous GI cells enables such a distinction. In addition, the panel distinguishes normal from cancerous cells.

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The invention thus comprises hybridoma cell lines producing mAbs recognizing human colon cancer cells, from the group of AS33, AS37, CLK314, CLH70, HT29/15, HT29/26, CLT307, CLT86, V-215, V-715. A preferred group comprises AS33, AS37, CLH70, CLK314, CLT86, CLT307, HT29-15, and HT29-26. These mAbs of the invention recognize colon or GI glycoprotein (gp) antigens molecular weights 25kd, 29kd and 95kd (mAbs CLH70, HT29/26 and CLT479 respectively). mAb CLT152 recognizes a protein antigen of 52 kd. The antigens for CLH6, CLT85, CLT174 and HT29/36 are heat stable and therefore probably glycolipids. CLT85, CLT479, CLT174, HT29/36, CLH68, CLT152 and HT29/15 are gamma sub one

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1 (gamma₁) immunoglobulins. HT29/26 is a gamma sub 2A
(gamma_{2A}) immunoglobulin. HT29/36 is a gamma sub 3 (gamma₃)
immunoglobulin and CLT218, CLT307, CLT86 and CLH6 are mu
immunoglobulins. (HT 29/36 is the same mAb as HT 29-36 or
5 29/36. HT 29/15 is the same mAb as HT 29-15 or 29/15 and HT
29/26 is the same mAb as HT 29-26 or 29/26. As 33 is the
same monoclonal antibody as A-33 and AS37 is the same as
A-37. In the tables below, CLH6 is the same mAb as 6, CLT86
is the same as 86, CLT85 is the same as 85, CLT307 is the
10 same as 307, CLT479 is the same as 479, CLT174 is the same
as 174, CLH 70 is the same as 70, CLT 15 is the same as 15,
CLH70 is the same as 70, CLT152 is the same as 152. The
following hybridoma cell lines and monoclonal antibodies
produced therefrom namely: HT 29/15, HT 29/26, HT 29/36, CLH
15 6 (or 6), CLT 85 (or 85), CLT 479 (or 479), CLT 174 (or
174), CLH68 (or 68), CLT 152 (or 152), CLH 70 (or 70) CLT
218 (or 218), CLT 15 (or 15), CLT 307 (or 307) and CLT 86
(or 86) have been disclosed and claimed in a previously
filed, co-pending application filed March 11, 1983, Serial
20 No. 474,415 herein incorporated by reference. These are
described in a publication Sakamoto et al _____ herein
incorporated by reference.

Description

25 A preferred embodiment of the present invention is to
test a human specimen as for example human body tissues,
wastes, fluids and exudates with each of the monoclonal

antibodies of the panel. The cells are tested or contacted separately with each of the monoclonal antibodies in a series of dilutions. Thus, an assay for cancer is possible with minimal patent disruption. Indeed, the present invention permits testing of human GI waste specimens for cell fragments containing antigenic markers for the monoclonal antibodies. Entire cells need not be present. Cytohistological methods requiring whole cells are not always successful.

The monoclonal antibodies of the present invention were prepared by the Kohler-Millstein procedure wherein spleen cells from a mouse (or other mammal) immunized with human cancerous colon cells or pancreas from established human tumor cell lines of fresh tumor tissue were fused with mouse myeloma to form hybridomas. By serological screening, antibodies from these hybridomas were found which recognize differentiation antigens on normal bladder and/or cancerous bladder. Other tissues, both normal and cancerous, may be recognized as well by some of these monoclonal antibodies. A system of classification of normal as well as cancerous colon based on these differentiation antigens is now possible, and serological assays for tumors of the colon have been developed. These assays are of special use in the early diagnosis of gastrointestinal cancer especially colon cancer.

TISSUE CULTURE:

Cultured human colon cancer cell lines came from Leibowitz and from the collection of J. Fogh at Sloan-Kettering Institute. Cultures of other established human cell lines and normal tissue cells have been described.

PRODUCTION OF MOUSE MONOCLONAL ANTIBODIES

BALB/c mice were immunized with either a colon carcinoma or pancreas carcinoma cell line or with fresh colon cancer tissues. Subcutaneous and intraperitoneal injections of 1×10^6 cells were given three to ten times at intervals of 2 weeks. Three days after the last injection, the fusion of immune spleen cells with mouse myeloma MOPC-21 NS/1 cells was performed as described. Culture supernatants were tested for antibody by the anti-mouse IG mixed hemmagglutination assay (MHA) or Protein A assay (PA) on a panel of cultured cell lines of colon and other types of tissue cells. After subcloning five to six times, hybridoma cells were injected subcutaneously into nu/nu mice (Swiss background) and sera from mice with tumors were collected and used for serological, immunopathological and biochemical characterization. In general these methods have been described in Ueda et al. (1981) Proc. Natl. Acad. Sci. U.S.A. 78:5122, Dippold et al. (1980) Proc. Natl. Acad. Sci. U.S.A. 77:6114.

1 SEROLOGICAL PROCEDURES:

5 The MHA, on cultured cells using rabbit anti-mouse Ig and mouse anti-SRBC has been described. Absorption tests, assessment of heat stability and proteinase sensitivity and antibody subclass determination were also performed as described. See Dippold et al, Supra and Ueda et al. Supra, Pfreundschuh et al. (1978) Proc. Natl. Acad. Sci. USA 75:5122, Ueda et al. (1979) J. Exp. Med. 150:564

10 IMMUNOPATHOLOGICAL PROCEDURES

Immunofluorescent staining of cryostat sections with fluorescein conjugated goat anti-mouse Ig (Cappel Laboratories) was performed as described. (Fradet et al. (1984) Proc. Natl. Acad. Sci USA January _____.

15 Immunoperoxidase staining, using monoclonal antibody, peroxidase conjugated goat anti-mouse Ig and 3-amino-9-ethylcarbazol (AEC) (Histoset, Ortho Diagnostic system) was carried out following procedures recommended by the manufacturer.

20 IMMUNOPRECIPITATION PROCEDURES:

25 Antibodies were tested for immunoprecipitation activity by using detergent solubilized cell extracts labeled by [³H] glucosamine. Nonidet P-40 solubilization of cells and

immunoprecipitation procedures using *Staphylococcus aureus* have been described. Aliquots of 2×10^6 [3H] cpm from unfractionated cell extracts were used. Precipitated molecules were extracted with 60 μ l of 0.01M Tris-HCl pH 7.2/2.0% NaDodSO₄/ 12.0mg of dithiothreitol per ml/15% (weight/volume) sucrose/0.01% pyronin Y by heating 5 min at 100°C and were analysed by polyacrylamide gel electrophoresis. Dippold et al. Supra. Cairncross et al., (1962) Proc. Natl. Acad. Sci. USA 79:5641.

The assay of the present invention comprises contacting a tissue containing colon cells with the antibody recognizing colon cell antigens, preferably monoclonal antibodies to one or more cell antigens of the gastrointestinal antigenic system, and observing the immunoserological or immunopathological antigenic reaction between said monoclonal antibody and said antigen. In a preferred embodiment of the invention, the tissue sample to be contacted is gastrointestinal tissue and the antigenic reaction of the contacted tissue is observed by well known techniques such as immunofluorescence, Rosette formation with sheep or human red blood cells linked to Protein A or anti-Ig direct absorption and the like. In another embodiment of the present invention, the tissue to be assayed is first excised and is then either freshly, or

1 after being frozen or embedded in paraffin by methods
well-known in the art, contacted with the monoclonal
antibodies of the invention. Observation of the reaction is
as before.

5 In another preferred embodiment of the present
invention, the tissue to be assayed comprises the intact
body of an individual or a whole portion thereof. The
antibody, tagged with a radioactive or other
10 energy-producing element, is administered to the individual,
and the whole body or part thereof is scanned externally for
localization of radioactivity at the site of cancerous
gastrointestinal cells. In another preferred embodiment
cancerous colon cells are located.

15 The present invention also makes possible the treatment
of gastrointestinal tumors in a patient wherein the
monoclonal antibody recognizing the cell antigen of
cancerous colon or other cancerous GI cells, is administered
20 to the patient in an amount effective to inhibit the growth
or proliferation of cancer cells. In a preferred embodiment
of this method, the antibody is tagged with a potentially
tissue destructive agent which causes destruction of the
cancer cells. Examples of tissue destructive agents
25 comprise chemotoxic agents, chemotherapeutic agents

including vaccines, radionuclides, toxins, complement
activators, clotting activators and the like. These
examples are for illustrative purposes only and are not
meant to limit the scope of the invention.

The following examples are intended to illustrate the
invention without limiting same in any manner especially
with respect to substantially functional equivalents of
hybridomas, monoclonal antibodies and cell lines described
and claimed herein.

The monoclonal antibodies selected for use in the
present invention were derived from spleen cells of mice
immunized with whole cells of colon carcinoma cell lines
such as Tallevi and HT-29 fresh tumor cell lines or
pancreatic tumor cell lines by fusion methods well known in
the art.

A group of monoclonal antibodies which were found to
recognize specific cell antigens of gastrointestinal cells,
was selected as the gastrointestinal panel. This panel and
other mAbs and the antigenic systems recognized are given in
Tables I, II, III and IV. Heterogeneity of human colon
carcinoma is therein noted. The table data point out and
define the heterogeneity of colon carcinomas.

1 Gastrointestinal antigenic systems are defined by these
mAbs as determined by serological analysis with over 70
tumor cell lines; 18 colon cancers, over 50
non-gastrointestinal cancers as well as immunopathology on
5 frozen sections of normal adult and normal fetal tissue and
cancer tissue. (See Table I, II, III and IV Reactivity with
tissue of cancer patients is shown in Table V)

10 Eight monoclonal antibodies to cell surface antigens of
human colon carcinoma were obtained by immunization with
cultured human colon and pancreas carcinomas or with lysates
of colon cancer cells. The distribution of the antigens
detected was analysed on 164 normal and malignant cell lines
(Table III) and on frozen sections of normal adult and fetal
15 tissues (Table IV). Fifty five colon carcinomas and normal
colonic tissue from the same patient were also examined
(Table V).

20 One very restricted antigen, V-215 (gp140), were
detected only on colon and four other cancer cell lines
(Table III). In several patients, the antigen were
expressed only on colon cancers but not in normal adjacent
colon tissues (Table V).

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1 A-33 antigen was found only on colonic and pancreatic
cancer cell lines, and not in any normal adult tissues, it
was present on both tumor and normal adjacent colonic
tissues (Tables III, IV and V).

5 K-314 antigen (gp170) was only on colon and a few lung
cancer cell lines (Tables III). In immunopathology, the
antigen was not found in any normal adult tissues except
some part of the proximal tubules of the kidney (Table IV).

10 A-37 antigen was on colon, some renal and hematopoietic
cell lines but was found only in the proximal tubules of
kidney in immunopathology staining (Tables III and IV).

15 HT-29-15 antigen (H-15) (Tables I-IV) was detected on
colon, breast and lung cancer cell lines and also, in
certain patients, was expressed on colon cancer tissues but
not on their normal counterparts (Table V). H-15
determinants are carried on a high molecular weight
glycoprotein and are neuraminidase-sensitive.

20 V-715 antigen (gp120) was expressed on colon, lung and
renal cancers (Tables III and IV). V-715 antigen has a
similar serological, and immunopathological characteristics
with the Adenosine deaminase protein. H-70 (Table I-IV)
25 antigen (gp29) was expressed on colon, lung, renal cancer
and neuroblastoma cell lines.

1 HT-29-26 antigen (gp31) (H-26) was detected on almost
all the epithelial tissues, but not in other tissues (Tables
I-IV).

5 None of the antigens were related to A, B, H, I or
Lewis blood group specificities.

Example I

10 Several of the antigens, as defined by the monoclonal
antibodies of the panel, are expressed differentially by
cell populations within the adult GI system. CLT152 antigen
is expressed by epithelial cells of the GI mucosa of
esophagus, stomach, small intestine and colon, but is not
found in other adult tissues. CLH70, CLT307, CLT86 and
15 CLH68 antigens are expressed by adult stomach, small
intestine and colon. CLT218 is expressed by adult small
intestine and colon. HT29/26 is expressed by colon and some
cells of small intestine in the adult. CLT15 also is
expressed by normal colon epithelium as well as some upper
20 GI cells except stomach in adult tissues. Thus the mAbs
antigens HT29/26, CLT15, CLT218, CLH70, CLT307, CLT86 and
CLH68 occur in adult colon epithelial cells; they vary among
themselves in their pattern of distribution within the rest
of the GI tract. There is some limited expression of these
25 antigens in epithelial cells of other tissues as well [See

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Table II]. Thus, for example, CLT218, CLT86, HT29/26 antigens are expressed on bronchial epithelium whereas CLH6, HT29/36 and HT29/15 are not. Thus, the panel antibodies differ in their expression on normal cells even as to similar cells of other tissues.

It is important that the mAbs CLH6, CLT85, and 29/36 do not react with normal adult tissue in immunopathology of frozen tissue sections but do react with distinct subsets of colon adenocarcinomas.

Serologically CLT85 reacts with approximately 11 of 17 colon cancer lines, and CLH6 with approximately 8 out of 17 colon cancer cell lines. CLT85 and CLH6 show no reaction with normal adult cells in serology.

From 23 further Köhler-Milstein fusions done as above of NS/1 myeloma with spleen cells, 8 more antibody producing clones were selected for further detailed analysis as discussed below. The serological specificities of these antibodies were tested on a panel of 154 established human cancer cell lines and on short term cultures of 10 human fibroblast and kidney epithelial cells (Tables III and IV). The immunopathological specificities of these antibodies were determined on a panel of human adult and fetal tissues, as well as normal colon epithelium and colon cancer tissues from 55 patients (Table V).

1 Monoclonal antibodies V-215, K-314 and V-715 were
obtained after immunization with fresh colon specimen;
antibodies A-33, A-37 were obtained after immunization with
pancreas cancer cell lines AsPc-1, and antibodies H-15, (HT
5 29/15 or HT29-15), H-70 and H-26 (HT 29/26 or HT29-26) were
obtained after immunization with colon cancer cell line
HT-29.

10 The heavy chain subclass of the eight antibodies are:
V-215, gamma-1; A-33, gamma-2b; K-314, gamma-1; A-37,
gamma-1; H-15, gamma-1; H-70, mu; H-26, gamma-2a.

Example II

15 V-215: Antibody V-215 react with 9/17 colon cancer cell
lines with a strongest titer of 5×10^4 against SW-1417 colon
cancer cell line by rosetting. One lung, one ovarian, one
terato-carcinoma and one melanoma cell lines were positive
but all 152 other cell lines tested were negative in direct
and absorption tests (Table III). The antigen was detected
20 on secretion of bronchial epithelium and uterine
endometrium, but not any other adult or fetal tissues tested
(Table IV). In immunoperoxidase staining of the frozen
section from 55 patients, V-215 was negative with normal but
positive with colon tumor in 7 patients (Table V).

1 V-215 antigen was immunoprecipitated from [³H]
glucosamine labelled cell extracts from colon cancer cell
line SW-1417. The molecular weight is 140000 as estimated
by polyacrylamide gel electrophoresis.

5

Example III

A-33: Antibody A-33 is an IgG2b antibody that reacts with
5/17 colon carcinomas and 1/3 pancreatic carcinoma (AsPc-1)
10 with a titer of 10. A-33 also reacts with 3/6 T cell
leukemia cell lines; all 155 other cell types tested were
negative. Correlation between A-33 and T cell related
antigens; OKT-6, T37,1, OKT-4, T,11, CL3-3 (13), CL3-40
(13), was examined by the inhibition tests and by rosetting
15 assay on immunizing cell line AsPc-1 and all the antigens
were negative in both tests.

Antibody A-33 react with normal adjacent colonic mucosa
and carcinoma of the colon cancer patient (Table V). One
20 out of 5 pancreatic mucosa and pancreas cancer of one
patient was also positive with the antigen. A-33 did not
react with any other tissue sections examined on
immunopathology staining (Table IV).

25

1 The antigen was not destroyed by heating at 100°C for 5
minutes and it was present in the chloroform/methanol
extract of AsPc-1 cells. In immunoprecipitation experiments
using cell extracts labelled with [³H]glucosamine,
5 radioactivity was precipitated that migrated at the dye
front in 9% acrylamide gels. These properties strongly
suggest that the antigen is a lipid.

EXAMPLE IV

10 K-314: Antibody K-314 reacted with 13/17 colon carcinomas
and 3/3 pancreas carcinomas, 7/25 lung carcinomas, 1/10
bladder carcinomas, and 3/5 chorio and teratocarcinomas; the
other 137 cell lines tested were negative (Table III).

15 In tissue sections, antibody K-314 reacted with lung,
uterus and heterogeneous population of gastrointestinal
tract epithelial cells of normal adult and fetal tissues
(Table IV). Among the gastrointestinal cancer patients,
20 K-314 is present in carcinoma but not in normal adjacent
mucosa in 11 colon cancer, 5 metastatic colon cancer to the
liver and 3 pancreas cancer patients (Table V).

25 K-314 was immunoprecipitated from [³H]glucosamine
labelled cell lysate of AsPc-1. The molecular weight of the
antigen is 170000 as estimated by polyacrylamide gel

1 electrophoresis.

EXAMPLE V

5 H-15: Antibody H-15 (HT-29-15) is an IgG1 antibody that
reacts with 12/17 colon cancers, 2/3 pancreatic cancers, 2/2
Hepatic and biliary cancers, 4/5 lung cancers, 1/8 bladder
cancers, 2/4 ovarian cancers and weak rosetting with one
melanoma and one renal cancer cell lines (Table III). H-15
is found in lung and in some proportion of gastrointestinal
10 mucosa in tissue sections (Table IV). H-15 is positive in
cancer but negative in normal counterpart of the same
patient in 8 colon cancers, 3 metastatic colon cancers and
in 2 pancreas cancers (Table V).

15 The antigen was not destroyed by heating at 100°C for 5
minutes and was proteinase resistant. The antigen
disappeared after treatment with neuraminidase. In
immunoprecipitation with [³H] glucosamine, weak broad band
of molecular weight over 200000 is observed.

Example VI

20 A-37: A-37 is present in 5/17 colon cancers and 3/3
pancreatic cancers, 3/20 renal cancers, 3/5 chorio- and
25 teratocarcinomas and in 15/25 hematopoietic cells tumors

1 (Table III). In tissue sections, the antigen was found only
on proxymal tubules of the kidney but not in any other
tissues tested (Table IV). This antigen is also heat
stable, proteinase and neuraminidase resistant.

5

Example VII

10 V-715: V-715 antigen is in 8/17 colon cancers, 5/25 lung
cancers, 1/10 bladder cancers and in almost all renal cancer
cell lines (Table III). In tissue sections, V-175 is
present in proxymal tubules of the kidney, but not in normal
gastrointestinal tract cells (Table IV). The antigen is
found on 9 colon and pancreas cancer specimen and on 4
normal adjacent colon and pancreas mucosa of those cancer
15 patients (Table V). The antigen is a glycoprotein and the
molecular weight is 120000.

20 The serology pattern with the cell lines and the
immunopathology staining pattern with the tissues are very
similar to the Adenosine deaminase binding protein which was
detected by renal cancer monoclonals (Andy, Robin J., et al.
(1984) J. Biol. Chem. 259:12844). Since V-175 is not
detected in normal colon, the determinant of V-715 is likely
to be the same as the epitope detected by monoclonal S-23.

25

Example VIII

1 H-70: H-70 is in 13/17 colon cancer cell lines and in
several other epithelial cancer cell lines. H-70 is also on
3/5 neuroblastoma cell lines. H-70 is detected in
5 epithelial tissues in immunopathology. Immunoprecipitation
with [H]glucosamine was performed to determine its molecular
weight as 31000.

Example IX

10 H-26: Antigen H-26 (HT-29-26,C-26) is in almost all
epithelial cancer cell lines but is not present in any
neuroblastoma, melanoma or astrocytoma cell lines (Table
III).

15 In immunopathology H-26 is present in all epithelial
cells and in kidney, it is present on distal and collecting
tubules (Table IV). H-26 is a glycoprotein and its
molecular weight is 29000.

20 Thus normal versus neoplastic cells of the colon, GI,
and pancreas can be differentiated and assayed by contacting
a specimen from a human patient with each of the monoclonal
antibodies of the panel in serial dilution, and observing
any antigen antibody reaction by any of the methods cited.

25 Although specific hybridomas producing monoclonal antibody

1 against gastrointestinal cell antigens are presented, it is
obvious that the present invention encompasses all the mAbs
exhibiting the characteristics described therein, especially
the embodiment describing reaction with normal as well as
5 tumor cell antigens of the GI tract.

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One of the preferred panels of the invention for colon cancer is:

SUMMARY OF MOUSE MONOCLONAL ANTIBODIES - COLON PANEL

Monoclonal Antibody (Ig subclass)	ATCC#	Molecular	Additional notes
AS33 (IgG2b)	HB 8779		N Colon/some Colon Ca.
AS37 (IgG1)	HB 8778		
CLH70 (IgG2b)	HB 8245	Mr29,000	
CLK314 (IgG1)	HB 8780		
CLT86 (mu)	HB 8252		
CLT307 (mu)	HB 8251		
HT29-15 (IgG1)	HB 8246	Mr 200,000	
HT29-26 (IgG2a)	HB 8247	Mr40,000	epithelial cell marker

Changes in cell antigens are associated with different stages of differentiation and different stages of cancer. Thus this invention technique defined cell antigens associated with differentiation and cancer of the GI tract and the pancreas.

Legend to Table I

Serological reaction of colon panel monoclonal antibodies with human tumor cell lines of various tissues by rosette formation with human red blood cells conjugated with

1 rabbit anti-Ig, Dippold Supra

where 0 = no reaction by rosette formation or
absorption

5 2 = positive rosette reaction at less than
1,000 fold dilution antibody
supernatant

10 3 = positive rosette reaction at greater
than 1,000 fold dilution antibody
supernatant

1 = positive reaction by absorption test
only.

15 If there is no rosette reaction, the absorption test was
done. Thus if a mAb was negative for the rosette reaction
but absorbed onto the test antigen system it was deemed to
be a positive reaction such that

20 1 = positive reaction by the absorption
test though mAb gives a negative test
for rosette formation

25 i.e. 0 test for rosette reaction is further tested by the
absorption test. Therefore 0 on this table indicates no
reaction by either absorption or rosette reactions. For

1 comparison, mAb 19.9 was obtained from H. Kaprowski and
assayed as well alongside the mAbs of the present invention
Atkinson, B.F. et al., Cancer Research, 42:4820-4823(1982).

5 In Table I actual titers are included.

Immunogen for CLT series is Tallevi, for HT and CLH
antibodies the immunogen is HT-29.

10 Legend to Table II

Immunopathological reaction of some of the colon panel
monoclonal antibodies with fetal and adult normal human
tissue and cancer tissue in frozen section by indirect
15 immunofluorescence.

0 = no reaction

⊙ = positive reaction

⊕ = heterogeneous reaction within the tissue

20 The following monoclonal antibody-producing-hybridoma
cell lines are maintained on deposit at Sloan-Kettering
Institute for Cancer Research, 1275 York Avenue, New York,
New York 10021 namely:

25 V-215, K-314, V-715, As-33, As-37, CLH 70, CLK
314, CLT 86, CLT 307, HT 29-15 and HT 29-26.

SerologySerological Reaction of Monoclonal Antibodies
Produced from Human Colon Tumor Immunogen With Various
Human Cancer Cell Lines

IMMUNIZING TUMOR: COLON

	CLH	CLT	CLT	CLT	CLH	CLT	CLH	HT29	HT29	HT29	CLT	CLT	CLT	CLT	
CELLS TESTED	6	85	479	174	68	152	70	-15	-26	-36	218	15	307	86	19.9
Liver Ca.:															
SK-HEP-1	1	0	0	0	0	0	2	3	3	0	0	0	0	0	0
Biliary duct:															
Charles	0	0	2	0	0	0	0	3	3		0	0		0	0
Astrocytoma:															
Goodstein	0	0	0	0	0	0	0	0			0	0	0	0	0
U251	0	0	0	0	0	0	0	0			0	0	0		0
Becker	0	0	0	0	0	0	0	0			0	0	0		0
Machino	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jones	0	0	0	0	0	0	0	0			0	0	0		0
AJ	0	0	0	0	0	0	0	0	0		0	0	0		0
Lear	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bealy	0	0	0	0	0	0	0	0	0		0	0	0	0	0
T98	0	0	0	0	0	0	0	0			0	0	0	0	0
U373	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melanoma:															
SK-MEL-31(3)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
-23	0	0	0	0	0	0	0	0	0		0	0	0	0	0
-13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-37	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
-93(2)	0	0	0	0	3	0	0	3	2	0	0	0	0	0	0
-28	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
MeWo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neuroblastoma:															
SK-N-MC	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
-SH	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
-BE2	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
LAN-1-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CHP-234N															

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Serology

Serological Reaction of Monoclonal Antibodies
Produced from Human Colon Tumor Immunogen With Various
Human Cancer Cell Lines

IMMUNIZING TUMOR: COLON

CELLS TESTED	CLH 6	CLT 85	CLT 479	CLT 174	CLH 68	CLT 152	CLH 70	HT29 -15	HT29 -26	HT29 -36	CLT 218	CLT 15	CLT 307	CLT 86	19.9
Breast Ca.:															
MDA MB 361	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0
MDA MB 231	0	0	0	0	0	0	2	3	3	0	0	0	3	0	0
BT 20	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0
CAMA	0	0	1	1	0	0	0	3	0		3	3	3	3	0
SK-BR-3	0	0	0	0	0	0	0	3	0		0	0	0	0	0
ALAB	0	0	0	0	0	0	3	3			0	0	0	0	0
MCF-7	0	0	0	0	0	0	0	3	3	3	3	0	0	3	0
Kidney Ca.:															
SK-RC-6	0	0	0	0	0	0	0	0	3	0	0	0	0	0	
-7	0	0	0	0	0	0	0	0	3	0					0
-29	0	0	0	0	0	3	0	0	3	0	0	0	0		0
-4	0	0	0	0	0	0	0	0	3	0	0	0	0		
Ovary Ca.:															
SK-OV-3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
FOAC	0	0	0	0	0	0	3	0	3		0	0	0	0	0
2774	0	0	0	0	0	0	0	0	3		0	0	0	0	0
SW 626	0	0	3	3	0	0	0	3	3		3	0	3	3	0
Snustak	0	0	0	0	0	3	0	3	3		3	3	3	3	0
Turanek	0		2	0	0	3	2	3	3	0	0	0	0	3	10
Uterine Ca.:															
ME180	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0
Chorioepithelium:															
SVOC	0	0	2		0	0	0	0	3		0	0	3	0	0

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Table I
Serology

Serological Reaction of Monoclonal Antibodies
Produced from Human Colon Tumor Immunogen With Various
Human Cancer Cell Lines

IMMINIZING TUMOR: COLON

CELLS TESTED	CLH	CLT	CLT	CLT	CLH	CLT	CLH	HT29	HT29	HT29	CLT	CLT	CLT	CLT	
	6	85	479	174	68	152	70	-15	-26	-36	218	15	307	86	19.9
Lung Ca:															
SK-LC-3	0	0	2	0	0	0	3	3	3	0	0	0	0	0	0
-4	0	0	0	0	0	0	0	3	3	2	0	0	0	0	10
-5	1	0	0	0	0	0	0	3	3	0	0	0	0	0	0
-6	0	0	0	0	0	0	3	2	0	0	0	0	2	0	0
-7	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0
-8	0	0	0	2	0	0	0	0	3	3	0	0	0	0	0
-13	0	0	0	0	0	0	2	0	3	2	0	0	0	0	0
Lawson	0	1	1	1	0	3	2	3	3	2	3	0	0	3	10 ³
Bladder Ca:															
T-24	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
TOC SUP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
253J	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
639V	0	0	0	0	0	0	0	0	3	3	0	0	0	0	0
486P	0	0	0	0	0	0	3	3	3	0	0	0	0	0	0

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Table II

Immunopathology

Normal Tissue Distribution of the Monoclonal
Antibodies Produced from Human
Colon Tumor Immunogen

A. FETAL TISSUES											
	CLT 85	CLH 6	HT 29/36	HT 29/15	CLT 479	CLT 15	CLT 174	CLT 86	CLH 70	CLT 152	19.9
LUNG	±	0	0	0	+	+	+	+	+	+	
Bronchial											
Epithelium	±	0	0	0	±	±	±	+	+	+	
Cartilage	0	0	0	0	0	0	0	0	0	0	
Pneumocytes	0	0	0	0	0	0	0	0	0	0	
Connect. Tis	0	0	0	0	0	0	0	0	0	0	0
HEART	0	0	0	0	0	0	0	0	0	0	0
THYMUS	0	0	0	0	+	0	0	0	0	+	+
Hassal's C..	0	0	0	0	+	0	0	0	0	+	+
Thymocytes	0	0	0	0	0	0	0	0	0	0	0
SPLEEN	0	0	0	0	0	0	0	0	0	0	0
White Pulp	0	0	0	0	0	0	0	0	0	0	0
Red Pulp	0	0	0	0	0	0	0	0	0	0	0
LIVER	0	0	0	0	0	0	0	+	+	+	0
	0	0	0	0	0	0	0	0	0	0	0
Biliary Epi											
Cells	0	0	0	0	0	0	0	+	+	+	0
GALLBLAD.	0	0	0	0	0	0	0	+	+	+	0
ESOPHAGUS	0	0	0	0	±	0	±	±	0	±	±
STOMACH	0	±	0	0	0	0	0	±	+	±	±
SMALL INT.	0	0	0	0	0	0	0	0	0	0	0
COLON	±	±	0	0	±	±	±	+	+	+	±
PANCREAS	0	+	0	+	+	+	+	+	+	+	+
Exocrine	0	±	0	+	+	+	+	+	+	+	+
Endocrine	0	0	0	0	0	0	0	0	0	0	0
KIDNEY	0	+	0	0	0	0	0	+	+	0	0
Glomerulus	0	0	0	0	0	0	0	0	0	0	0
Prox. Tub.	0	0	0	0	0	0	0	0	+	0	0
Distal Tub.	0	+	0	0	0	0	0	+	0	0	0
Collec. Tub	0	+	0	0	0	0	0	+	0	0	0
URETER	0	+	0	±	0	0	0	±	0	+	0
UR. BLAD.	0	+	0	±	0	0	0	±	0	+	0
ADRENAL	0	0	0	0	0	0	0	0	0	0	0
Cortex	0	0	0	0	0	0	0	0	0	0	0
Medulla	0	0	0	0	0	0	0	0	0	0	0
TESTES	0	0	0	0	0	0	0	0	0	0	0
Germ. Cells	0	0	0	0	0	0	0	0	0	0	0
Endoc. Cel.	0	0	0	0	0	0	0	0	0	0	0
Connect. T.	0	0	0	0	0	0	0	0	0	0	0

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TABLE II

ImmunopathologyNormal Tissue Distribution of the Monoclonal
Antibodies Produced from Human
Colon Tumor Immunogen

A. FETAL TISSUES (Cont'd.)

	CLT		CLH	HT	HT	CLT	CLT	CLT	CLT	CLH	CLT	
	85	28	6	29/36	29/15	479	15	174	86	70	152	19.9
ADIPY	0	0	0	0	0	0	0	0	0	0	0	0
Serm. Cells	0	0	0	0	0	0	0	0	0	0	0	0
Connect. T.	0	0	0	0	0	0	0	0	0	0	0	0
FALLOP. T.	0	0	0	0	0	0	0	0	0	0	0	0
UTERUS	0	0	0	0	0	0	0	0	+	+	0	0
Endometrium	0	0	0	0	0	0	0	0	+	+	0	0
Myometrium	0	0	0	0	0	0	0	0	0	0	0	0
CERVIX	0	0	0	0	0	0	0	0	+	0	0	0
Endocervix	0	0	0	0	0	0	0	0	+	0	0	0
Exocervix	0	0	0	0	0	0	0	0	±	0	0	0
SKIN	0	0	0	0	0	0	±	0	+	0	+	0
Epidermis	0	0	0	0	0	0	±	0	±	0	±	0
Melanocytes	0	0	0	0	0	0	0	0	0	0	0	0
Sweat Gland	0	0	0	0	0	0	0	0	0	0	0	0
Seb. Gld.	0	0	0	0	0	0	0	0	0	0	0	0
Hair Fol.	0	0	0	0	0	0	0	0	0	0	0	0
Dermis C.T.	0	0	0	0	0	0	0	0	0	0	0	0
BRAIN	0	0	0	0	0	0	0	0	0	0	0	0
Neurons	0	0	0	0	0	0	0	0	0	0	0	0
Glial Cells	0	0	0	0	0	0	0	0	0	0	0	0
Dendrites	0	0	0	0	0	0	0	0	0	0	0	0
LYMPH NODE	0	0	0	0	0	0	0	0	0	0	0	0
BLOOD VES.	0	0	0	0	0	0	0	0	0	0	0	0
Endoth. Cel.	0	0	0	0	0	0	0	0	0	0	0	0
Smooth Ms.	0	0	0	0	0	0	0	0	0	0	0	0
SOFT TIS.	0	0	0	0	0	0	0	0	0	0	0	0
SECRETION	±	0	0	0	0	+	0	+	+	+	+	+

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ImmunopathologyNormal Tissue Distribution of the Monoclonal
Antibodies Produced from Human
Colon Tumor Immunogen

A. FETAL TISSUES (CONT'D.)

	CLT 307	CLT 218	HT 29/26	CLH 68
LUNG	+	+	+	0
Bronchial Epithelium	+	+	+	0
Cartilage	0	0	0	0
Pneumocytes	0	0	0	0
Connect. Tis	0	0	0	0
HEART	0	0	0	0
THYMUS	0	0	0	+
Hassal's C.	0	0	0	+
Thymocytes	0	0	0	0
SPLEEN	0	0	0	0
White Pulp	0	0	0	0
Red Pulp	0	0	0	0
LIVER	+	+	+	+
Hepatocytes	0	0	0	0
Biliary Epi Cells	+	+	+	+
GALLBLAD.	+	+	+	+
ESOPHAGUS	+	±	±	±
STOMACH	±	+	0	+
SMALL INT.	0	0	±	+
COLON	+	+	+	+
PANCREAS	+	+	+	0
Exocrine	+	+	+	0
Endocrine	0	0	0	0
KIDNEY	0	+	+	0
Glomerulus	0	0	0	0
prox. Tub.	0	0	0	0
Distal Tub.	0	+	+	0
Collec. Tub	0	+	+	0
URETER	+	+	+	+
UR. BLAD.	+	+	+	+
ADRENAL	0	0	0	0
Cortex	0	0	0	0
Medulla	0	0	0	0
TESTES	0	0	0	0
Germ. Cells	0	0	0	0
Endoc. Cel.	0	0	0	0
Connect. T.	0	0	0	0

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0195141

TABLE IIA
SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST
COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

SK 340
4/12/85

MONOCLONAL ANTIBODIES															
NORMAL FETAL TISSUES	85	28	6	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
KIDNEY															
Glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proximal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Collecting Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URETER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URINARY BLADDER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ADRENAL															
Cortex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TESTES															
Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocrine Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OVARY															
Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FALLOPIAN TUBES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UTERUS															
Endometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERVIX															
Endocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE IIA

SK 840
4/12/85

SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST
COLON CANCER BY IMMUNOPATHOLOGICAL IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

0199141

NORMAL FETAL TISSUES		85	29	5	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
LUNG		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bronchial Epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cartilage		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pneumocytes		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HEART		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THYROID		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hassall's Corpuscles		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymocytes		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SPLEEN		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
White pulp		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red pulp		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIVER		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hepatocytes		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biliary Epith. Cells		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GALLBLADDER		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ESOPHAGUS		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
STOMACH		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SMALL INTESTINE		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COLON		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PANCREAS		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exocrine		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocrine		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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MONOCLONAL ANTIBODIES

[illegible]

TABLE IIA

SK 340
4/12/85

SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST
COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

NORMAL ADULT TISSUE	MONOCLONAL ANTIBODIES															
	85	28	6	29/36	29/15	479	15	174	86	70	152	19.9	307	218	29/26	68
KIDNEY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proximal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Henle's Loop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Collecting Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URETER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
URINARY BLADDER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ADRENAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cortex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
THYROID	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colloid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BREAST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Duct Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acinar Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PROSTATE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stroma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TESTES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocrine Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table IIA

SK 340
4/12/85SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST
COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

MONOCLONAL ANTIBODIES

NORMAL ADULT TISSUES	85	18	6	29/36	29/15	479	15	174	86	70	152	19.9	307	218	29/26	61
OVARY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T. Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O:Connective Tissue	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FALLOPIAN TUBES	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UTERUS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CERVIX	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLACENTA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cytotrophoblasts	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Syncytiotrophoblasts	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sinusoids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SKIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epidermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melanocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sweat Gland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sebaceous Gland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dermis Connective Tis.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRAIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neurons	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glial Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dendrites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ADON HPHAT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

TABLE IIA

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	85	26	6	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
CANCER TISSUE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
COLON CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LUNG CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BREAST CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BLADDER CANCER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TERATOGENESIS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MELANOMA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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SUMMARY OF TISSUE DISTRIBUTION AND REACTIVITY OF MONOCLONAL ANTIBODIES AGAINST COLON CANCER BY IMMUNOPATHOLOGY IN NORMAL, FETAL, NORMAL ADULT AND CANCER TISSUE IN HUMAN SPECIMENS

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TABLE IIA

MONOCLONAL ANTIBODIES

	85	28	6	29/36	29/15	479	15	174	86	70	152	307	218	29/26	68
1-41 CANCER TISSUE															
019 ASTROCYTOMA	0	0	0		0	0	0	0	0		0			0	
	0		0		0	0	0	0	0		0			0	
LYMPHOMA	0	0	0		0	0	0	0	0		0			0	
	0		0		0	0	0	0	0		0			0	
KIDNEY CANCER	0	0	0		0	0	0	0	0		0			0	
	0		0		0	0	0	0	0		0			0	
	0		0		0	0	0	0	0		0			0	
	0		0		0	0	0	0	0		0			0	
	0		0		0	0	0	0	0		0			0	

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TABLE III REACTIVITY OF MOUSE MONOCLONAL ANTIBODIES GENERATED AGAINST COLORECTAL CANCERS.
SEROLOGICAL TEST WITH CULTURE HUMAN CELLS

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CELLS	V-115	A-33	K-314	A-37	H-15	V-715	H-70	H-26
Immunoglobulin subclass	Y-1	Y-2b	Y-1	Y-1	Y-1	Y-1	Y-1	Y-2a
Molecular weight	9P 140		9P 170			9P 120	9P 31	9P 29

EPITHELIUM ORIGIN TUMORS

COLON

HT-29, SW-480, SW-403	0	0	0	0	0	0	0	0
SW-48, CACO-2, SW-1116	0	0	0	0	0	0	0	0
SW-CO-19, SK-CO-13	0	0	0	0	0	0	0	0
SW-1417, SW-12372, SK-CO-15	0	0	0	0	0	0	0	0
SW-620, SW-817, SK-CO-11	0	0	0	0	0	0	0	0
SW-1083, SK-CO-12, SK-CO-1	0	0	0	0	0	0	0	0

PANCREAS

ASP-C-1, CAPAN-1, CAPAN-2

ASP-C-1, CAPAN-1, CAPAN-2	0	0	0	0	0	0	0	0
---------------------------	---	---	---	---	---	---	---	---

HEPATIC AND BILIARY

SK-HEP-1, SK-HPL-2

SK-HEP-1, SK-HPL-2	0	0	0	0	0	0	0	0
--------------------	---	---	---	---	---	---	---	---

LUNG

J-82, CALU-1, CALU-5
CALU-6, SK-MES-1, SK-LU-1
SK-LC-1, -2, -4
SK-LC-5, -6, -9
SK-LC-8, -10, -12
SK-LC-15, -16, -17
SK-LC-18, -19, -23
SK-LC-24, -25, -28
SK-LC-L1

J-82, CALU-1, CALU-5	0	0	0	0	0	0	0	0
CALU-6, SK-MES-1, SK-LU-1	0	0	0	0	0	0	0	0
SK-LC-1, -2, -4	0	0	0	0	0	0	0	0
SK-LC-5, -6, -9	0	0	0	0	0	0	0	0
SK-LC-8, -10, -12	0	0	0	0	0	0	0	0
SK-LC-15, -16, -17	0	0	0	0	0	0	0	0
SK-LC-18, -19, -23	0	0	0	0	0	0	0	0
SK-LC-24, -25, -28	0	0	0	0	0	0	0	0
SK-LC-L1	0	0	0	0	0	0	0	0

BLADDER

253-J, SW-780, FTCSUP
5637, VM-CUB-1, VM-CUB-2
VM-CUB-3, 575-A, RT-4
638-V

253-J, SW-780, FTCSUP	0	0	0	0	0	0	0	0
5637, VM-CUB-1, VM-CUB-2	0	0	0	0	0	0	0	0
VM-CUB-3, 575-A, RT-4	0	0	0	0	0	0	0	0
638-V	0	0	0	0	0	0	0	0

ECTODERM ORIGIN TUMORS

BREAST

HDA-MB-361, MCF-7, CAMA
SK-BR-3, MDA-MB-157, ATAB

HDA-MB-361, MCF-7, CAMA	0	0	0	0	0	0	0	0
SK-BR-3, MDA-MB-157, ATAB	0	0	0	0	0	0	0	0

ASTROCYTOMA

NEUROBLASTOMA

MESODERM ORIGIN TUMOR

SK-RC-1,-2,-4
SK-RC-7,-9,-10
SK-RC-17,-18,-21
SK-RC-26A,-26B,28
SK-RC-29,-35,-37
SK-RC-39,-42,-44
SK-RC-45,-48

B CELL

T CELL

MONOCYTE

NULL CELL

NALM-1, NALM-16, NKL 1
NKL 2, NALL 1

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REACTIVITY OF ANTIBODIES GENERATED AGAINST GASTROINTESTINAL
CANCERS: IMMUNOFLOUORESCENCE TESTS WITH FROZEN SECTIONS OF NORMAL
HUMAN FETAL (F)* AND ADULT (A) TISSUES

Normal Human Tissues	V-215		A-33		K-314		A-37		H-15		V-715		H-70		H-2	
	F	A	F	A	F	A	F	A	F	A	F	A	F	A	F	A
Colon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Esophagus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pancreas-Endocrine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Exocrine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver-Hepatocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Biliary epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung-Bronchial epithelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Pneumocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endothelium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prostate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney-Glomerulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Proximal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Henle's Loop	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Distal Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Collecting Tubules	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testis-Germ Cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Endocrine cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ovary	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Placenta-Syncytiotrophoblast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Cytotrophoblast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uterus-Endometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Myometrium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cervix-Endocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Exocervix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Breast-Duct cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Acinar cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin-Epidermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Adnexa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Melanocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brain-Neurons	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Glial cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Dendrites	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thyroid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spleen-White Pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-Red Pulp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymph Nodes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Endothelial cells	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibroblasts	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cartilage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Interstitial Matrix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Secretions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Reactivity of monoclonal antibodies with tissue sections is symbolized as follows:
0, no immunofluorescence; ●, immunofluorescence, ○, heterogenous pattern of immunofluorescence.

*Fetal tissues were obtained from a 14 weeks old fetus.

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TABLE V REACTIVITY OF MONOCLONAL ANTIBODIES WITH THE TUMOR AND NORMAL ADJACENT FROZEN TISSUE SECTIONS OF CANCER PATIENT

Location	Patient	Monoclonal antibodies					
		V-215	A-33	K-313	H-15	V-715	H-26
Rectal colon	AR	-	+	-	-	-	+
	AY	-	+	⊙	+	-	+
Sigmoid colon	BR	-	+	+	⊙	-	+
	BT	-	+	+	+	-	+
	CH	⊙	+	+	⊙	⊙	+
	CR	-	+	+	+	-	+
	DE	-	+	⊙	-	-	+
	DN	⊙	+	⊙	⊙	⊙	+
	FA	-	+	⊙	-	⊙	+
	FU	-	+	⊙	-	-	+
	GA	-	+	⊙	-	+	+
	GP	-	+	+	+	-	+
	GY	⊙	+	⊙	+	-	+
	HT	⊙	+	⊙	⊙	-	+
	HK	-	+	+	+	-	+
	MS	-	+	⊙	+	-	+
	NC	-	+	+	-	-	+
	ND	-	-	-	-	-	+
	OT	-	+	+	⊙	⊙	+
	PP	-	-	+	-	-	+
	RD	-	+	⊙	+	-	+
	RT	-	+	⊙	+	-	+
	RV	-	+	+	-	-	+
	VC	-	+	+	-	-	+
Left colon	BW	-	+	+	⊙	-	+
	HL	⊙	+	⊙	+	-	+
	MT	-	+	+	-	⊙	+
	SM	-	-	+	-	-	+
	ST	-	+	+	-	-	+
Right colon	BA	⊙	+	+	-	-	+
	BG	-	+	+	-	-	+
	PS	-	+	⊙	⊙	-	+
	GA	-	+	+	+	-	+
	HA	-	+	+	-	-	+
	HU	+	+	+	-	-	+
	KP	-	-	⊙	⊙	-	+
	SH	-	-	-	-	-	+

1 What is Claimed:

- 5 1. Monoclonal antibodies characterized by immunological binding to human gastro-intestinal cell antigens and wherein said monoclonal antibody is selected from the group consisting of V-215, K-314, V-715, AS-33, and AS-37.
- 10 2. Monoclonal-antibody-producing-hybridoma cell line formed by fusing a myeloma cell line and spleen cells derived from a mammal immunized with established culture cell lines of human gastrointestinal cell carcinomas, pancreatic tumor cell lines, wherein the monoclonal antibody is selected from the group
- 15 consisting of monoclonal antibody V-215, K-314, V-715, AS-33 and AS-37.
- 20 3. Panel of monoclonal antibodies for the diagnosis of human gastrointestinal cancer wherein the panel consists of two or more different monoclonal antibodies selected from the group consisting of V-215, K-314, V-715, AS-33, and AS-37.
- 25

- 1 4. Panel of claim 3 wherein the human gastrointestinal
cancer diagnosed is human colon cancer.
- 5 5. Panel of monoclonal antibodies for the diagnosis of
human gastrointestinal cancer wherein the panel
consists of two or more different monoclonal antibodies
selected from the group consisting of AS33, AS37,
V-214, V-715, CLH70, CLK314, CLT86, CLT307, HT 29-15,
and HT 29-26.
- 10 6. Panel of claim 5 wherein the human gastrointestinal
cancer diagnosed is human colon cancer.
- 15 7. Method for differentiating normal and abnormal
gastrointestinal cells which comprises contacting a
human gastrointestinal specimen containing
gastrointestinal cellular material with two or more of
the monoclonal antibodies of from the group consisting
of AS-33, AS-37, V-215, V-715, CLH70, CLK 314, CLT86,
20 CLT307, HT29-15 and HT 29-26 and detecting the presence
or absence of immune complex formation with two or more
of said monoclonal antibodies indicating the presence
or absence of abnormality in the gastrointestinal
specimen.
- 25

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8. Method of Claim 7 wherein the abnormality is gastro-intestinal cancer.
- 5
9. Method of Claim 7 wherein the abnormality is colon cancer.
- 10
10. Method of Claim 7 wherein the specimen is contacted singly, serially or in combination with each of hte panel monoclonal antibodies.
- 15
- 20
- 25
- 30

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EUROPEAN PATENT APPLICATION

①⑰ Application number: **86104321.4**

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C 12 P 21/00, C 12 N 5/00
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⑥④ **Monoclonal antibodies to human gastrointestinal cancer.**

⑤⑦ Diagnostic panels for human gastrointestinal abnormalities such as cancer using mouse monoclonal antibodies are disclosed. These panels can be used in diagnosis and in therapeutic applications such as colon cancer.



European Patent
Office

EUROPEAN SEARCH REPORT

0199141

Application Number

EP 86 10 4321

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
D, X	EP-A-0 119 556 (SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH) * Claims; page 7, lines 13-17 * -----	1-7	C 07 K 15/00 C 12 P 21/00 C 12 N 5/00 C 12 N 15/00 G 01 N 33/577 G 01 N 33/573// (C 12 P 21/00 C 12 R 1:91)
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			C 12 P C 12 N G 01 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 25-03-1988	Examiner RYCKEBOSCH A.O.A.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document			